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Structural Changes in Acetylcholinesterase under the Influence of Some Ligands

Miloš R. Pavlič

Institute of Biochemistry and Institute of Pathophysiology, Medical Faculty, University of Ljubljana, 61000 Ljubljana, Yugoslavia

In order to elucidate the nature of the effect of some ligands on the methanesulfonylation of acetylcholinesterase (acetylcholine acetylhydrolase, EC 3.1.1.7), the methanesulfonylation of the enzyme was studied, in the absence and presence of ligands, at various temperatures and at various dielectric constants. The thermodynamic quantities obtained for the overall reaction, $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$, and $\Delta G^{\ddagger}$, point to specific structural changes in the esteratic site of the enzyme under the influence of accelerators of methanesulfonylation but do not clearly answer the question about the nature of the effect of the ligands. A closer analysis of the activation entropy for the reaction in the absence and presence of decamethonium indicates that the acceleration effect of this ligand on the methanesulfonylation of acetylcholinesterase is an electrostatic effect and is associated with a relatively less favourable conformational change in the esteratic site of the enzyme.

INTRODUCTION

Since 1963 when Kitz and Wilson[1] found that tetraethylammonium and some other ligands accelerate the methanesulfonylation of acetylcholinesterase (acetylcholine acetylhydrolase, EC 3.1.1.7) many workers have been dealing with this interesting acceleration phenomenon.

We investigated the influence of tetraethylammonium and some acetylcholine-receptor activating and blocking agents on the methanesulfonylation of acetylcholinesterase[2,3]. We found that tetraethylammonium and the tested activating agents, decamethonium and carbamylcholine, accelerated methanesulfonylation by increasing the activation entropy of the reaction. This indicates that these ligands give rise to a structural change in the enzyme. The tested blocking agents, gallamine and D-tubocurarine, had little influence on methanesulfonylation. Consequently, they probably have no influence on the structure of acetylcholinesterase.

Since a receptor activating agent is supposed to cause a structural change in the receptor whereas a blocking agent does not do so, the correlation of the effects of these ligands on acetylcholinesterase and on the acetylcholine-receptor points to a similarity between or even identity of, acetylcholinesterase and acetylcholine-receptor. Roufogalis and Wickson[4], however, showed that both decamethonium and gallamine accelerated the carbamylation of acetylcholinesterase by o-nitrophenoldimethylcarbamate and that there was no correlation between the obtained thermodynamic quantities and the kinetic effects of these ligands on carbamylation. It seems therefore worthwhile to

look for a possible correlation beyond the acceleration and activation entropy level.

No matter whether there is such a correlation or not a closer inspection of the nature of the accelerating effect was thought to yield interesting information. We therefore analysed the methanesulfonylation of acetylcholinesterase with a treatment which makes it possible to distinguish between the contribution of the structure and of the solvent effect to the activation entropy of the reaction. As a test ligand decamethonium was chosen since it is a good accelerator and also a strong activator.

METHODS AND MATERIALS

The methanesulfonylation of acetylcholinesterase proceeds according to the following scheme[5,6]

$$EH + I \xrightarrow{k_a} E' + HF \qquad (1)$$

where EH is the esteratic site of the enzyme, I the inhibitor methanesulfonyl fluoride, E′ the acylated enzyme, HF the leaving hydrogen fluoride, and k_a the second-order rate constant for methanesulfonylation. It seems that the raction involves a Michaelis-Menten complex, although it is not easy to show this. The corresponding scheme is[7]

$$EH + I \overset{K_i}{\rightleftharpoons} EHI \xrightarrow{k_2} E' + HF \qquad (2)$$

where EHI is the Michaelis-Menten complex between the esteratic site and methanesulfonyl fluoride, K_i the Michaelis-Menten constant, and k_2 the first-order rate constant for the decomposition of the Michaelis-Menten complex; the second order rate constant for the overall reaction is k_a and equals k_2/K_i.

The acceleration effect of decamethonium on methanesulfonylation was studied by determining the second-order rate constant for the methanesulfonylation of acetylcholinesterase in the presence and absence of decamethonium and at various dielectric constants; the obtained results were analysed according to Barnard and Laidler[8].

Each second-order rate constant was determined essentially as already described[2,6]. The enzyme was incubated with a certain concentration of methanesulfonyl fluoride for various lengths of time, the remaining activity determined according to the method of Hestrin[9] or Ellman et al.[10], and the (apparent) first-order rate constant calculated from the time dependence of methanesulfonylation; three first-order rate constants were always determined for three different concentrations of methanesulfonyl fluoride. The second-order rate constant was then determined from the dependence of the first-order rate constant on the concentration of methanesulfonyl fluoride. The concentration of methanesulfonyl fluoride ranged from 0.2 mM to 2 mM; in experiments with decamethonium the concentrations were ten times lower. The concentration of decamethonium ranged from 2 μM to 200 μM; from the dependence of the second-order rate constant on the concentration of decamethonium the maximum value of this constant was extrapolated and used for further calculation. For changing the dielectric constant of the medium methanol was used in concentrations up to 20%. In the determination of the first-order rate constants the slight inactivation of the enzyme at higher concentrations of methanol was taken into account.

Subsequently, the logarithms of the second-order rate constants at different dielectric constants were plotted against the corresponding reciprocals of the dielectric constants, $1/\varepsilon$, and the electrostatic activation entropies for methanesulfonylation, $\Delta S^{\ddagger}_{e.s.}$, obtained by means of equations.

$$\ln k_a = \ln (k_a)_o + \frac{A}{\varepsilon T} \qquad (3)$$

$$\Delta S^{\ddagger}_{e.s.} = 1.13 \times 10^{-4} A \qquad (4)$$

Finally, the noneelectrostatic activation entropies, $\Delta S^{\ddagger}_{\text{n. e. s.}}$, were calculated by subtracting the electrostatic activation entropies from the overall activation entropies, $\Delta S^{\ddagger}$, determined from the temperature dependence of methanesulfonylation[2,3].

All experiments were carried out at 25 °C. The universal buffer solution of Britten and Robinson[11] was used; sodium chloride was added to obtain a total ionic strength of 0.2 M; the pH of the buffer was 8.4 which is the optimum value for the methanesulfonylation of acetylcholinesterase at 25 °C.

The enzyme used was acetylcholinesterase from the electric organ of *Electrophorus electricus*, Worthington, ECHP 53N514, 1300 u/mg. The stock solution of the enzyme contained 0.1 mg of the preparation in 1 ml of the universal buffer solution of Britten and Robinson, pH = 8.4, ionic strength 0.2. Methanesulfonyl fluoride was from Eastman Organic Chemicals. The stock solution of methanesulfonyl fluoride was a 50 mM solution of the inhibitor in spectroquality acetone. Eeach incubation solution was prepared immediately before the experiment.

RESULTS AND DISCUSSION

The overall activation entropies for the methanesulfonylation of acetylcholinesterase in the absence and presence of decamethonium[2,3] as determined from the temperature dependence of methanesulfonylation are — 16 and — 10 cal K^{-1} mol^{-1}, respectively. The difference between the two values, + 6 cal K^{-1} mol^{-1}, indicates a structural change in the esteratic site of acetylcholinesterase under the influence of decamethonium.

Fig. 1 shows the dependence of the logarithm of the second order rate constant for methanesulfonylation of acetylcholinesterase on the reciprocal of the dielectric constant in the absence and presence of decamethonium. It can be seen from the diagram that the logarithm decreases with the increasing reciprocal of the dielectric constant and that the dependence is strictly linear, which is in accordance with equation (3). This indicates that the effect of methanol is in accordance with the theory of the influence of the dielectric constant on the rate of a reaction. The action of a solvent on a reaction, however, may be rather diverse and the above accordance only a coincidence (*cf.* ref. 12).

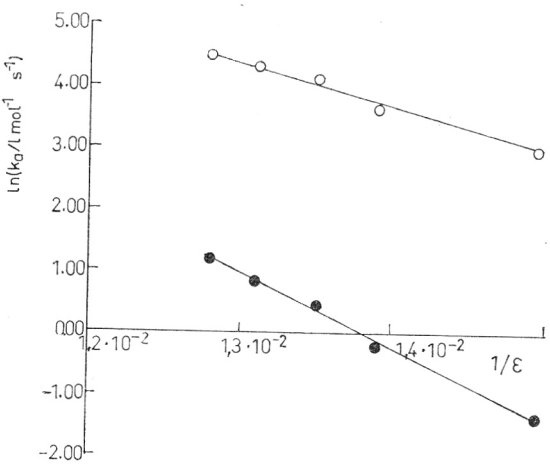

Fig. 1. The dependence of the logarithm of the second-order rate constant, k_a, for the methanesulfonylation of acetylcholinesterase on the reciprocal of the dielectric constant, $1/\varepsilon$, in absence (●) and presence (○) of decamethonium.

TABLE I.

The activation entropies (at 25 °C) for the methanesulfonylation of acetylcholinesterase in the absence and presence of decamethonium. $\Delta S^{\ddagger}$, overall activation entropy; $\Delta S^{\ddagger}_{e.s.}$, electrostatic activation entropy; $\Delta S^{\ddagger}_{n.e.e.}$, nonelectrostatic activation entropy; $\Delta\Delta S^{\ddagger}$, change in the activation entropy under the influence of decamethonium

Activation entropy:	in the absence of decamethonium	in the presence of decamethonium
$\Delta S^{\ddagger}$/cal K^{-1} mol^{-1}	— 16	— 10
$\Delta S^{\ddagger}_{e.s.}$ /cal K^{-1} mol^{-1}	— 40	— 23
$\Delta S^{\ddagger}_{n.e.s.}$ /cal K^{-1} mol^{-1}	+ 24	+ 13
$\Delta\Delta S^{\ddagger}$/cal K^{-1} mol^{-1}	+ 6	
$\Delta\Delta S^{\ddagger}_{e.s.}$ /cal K^{-1} mol^{-1}	+ 17	
$\Delta\Delta S^{\ddagger}_{n.e.s.}$ /cal K^{-1} mol^{-1}	— 11	

The values of the electrostatic and nonelectrostatic activation entropy (Table I) in the absence of decamethonium are very illustrative. The high negative value of — 40 cal K^{-1} mol^{-1} for the electrostatic activation entropy indicates a considerable charge separation during the course of the reaction; since the value is so high the charge separation seems to be associated with the binding of water (*cf.* ref. 13). A charge separation during the course of the acylation of acetylcholinesterase is consistent with the accepted mechanism of the acylation of this enzyme (*cf.* ref. 14). The large positive value of the nonelectrostatic activation entropy, + 24 cal K^{-1} mol^{-1}, indicates a favourable conformational change during methanesulfonylation.

The values of the activation entropy for the reaction in the presence of decamethonium (Table I) speak again for a charge separation and a favourable small conformational change in the enzyme during the reaction.

The effect of decamethonium is reflected in a change in the activation free energy for the transition from the ground state to either the activated complex for the formation of the Michaelis-Menten complex or to the activated complex for the formation of the acylated enzyme (*cf.* ref. 15). The corresponding changes in the activation entropy, $\Delta\Delta S^{\ddagger}$, are considerable (Table I). It is evident from these values that, in the presence of decamethonium, a less favourable conformational change is more than compensated for by a less unfavourable electrostatic process. These findings suggest that the acceleration effect of decamethonium on the methanesulfonylation of acetylcholinesterase is an electrostatic effect of the ligand on methanesulfonylation (*cf.* ref. 16) and that this effect is associated with a less favourable conformational change in the esteratic site of the enzyme.

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DISCUSSION

T. L. Rosenberry:

Can one separate the thermodynamic effects on k_2, the first-order rate constant of methanesulphonylation, from the initial pre-equilibrium constant K_i?

M. R. Pavlič:

Yes, and this would give information about the details of the mechanism. However, it is not easy to do that, since the concentration of the enzyme-inhibitor complex is very low. About seven years ago Wilson and I obtained a value for K_i of about 0.2 M, but did not publish the result because we felt that the value was not reliable; a similar value for K_i was obtained by Iverson (*Mol. Pharmacol.* **7** (1971) 129). As far as I know these are the only two determinations.

IZVLEČEK

Strukturne spremembe v acetilholinesterazi pod vplivom nekaterih ligandov

M. R. Pavlič

Namen našega dela je bil razjasniti naravo vpliva nekaterih ligandov na metansulfoniliranje acetilholinesteraze (acetilholin acetilhidrolaze; EC 3.1.1.7). Proučevali smo metansulfoniliranje encima, brez dodanih ligandov in z njimi, pri različnih temperaturah in pri različnih dielektričnih konstantah. Dobljene termodinamične količine za reakcijo, $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$ in $\Delta G^{\ddagger}$, govore za specifične spremembe v strukturi esteraznega mesta acetilholinesteraze pod vplivom akceleratorjev metansulfoniliranja, ne dajo pa jasnega odgovora na vprašanje o naravi vpliva ligandov. Podrobnejša analiza aktivacijske entropije za reakcijo v navzočnosti dekametonija in brez njega kaže, da je akceleracijski efekt tega liganda na metansulfoniliranje acetilholinesteraze elektrostatičen efekt in da je povezan z razmeroma manj ugodno konformacijsko spremembo v esteraznem mestu encima.

INŠTITUT ZA PATOLOŠKO FIZIOLOGIJO
MEDICINSKA FAKULTETA
UNIVERZA V LJUBLJANI
61105 LJUBLJANA